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Artigo Científico
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Proposal for qualitative and quantitative descriptors to characterise bamboo germplasm[1]

Proposta de descritores qualitativos e quantitativos para caracterização de germoplasma de bambu

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ABSTRACT - Bamboo is a genetic resource with evident potential for use from construction until the recovery of degraded areas. Although, characterization and evaluation studies involving bamboo species are scarce and it is difficult to define a list of specific descriptors to better meet the different genres and or bamboo species. Thus, the objective of this work were propose and test the effectiveness of qualitative and quantitative descriptors for phenotypic characterization and the study of genetic diversity among six species of bamboo introduced in Brazil. Fifteen qualitative and nine quantitative descriptors were proposed and tested in clones with one year of planting. Individuals belonging to six species of bamboo (*Bambusa vulgaris*, *Bambusa vulgaris* var. *vittata*, *Drepanostachyum falcatum*, *Dendrocalamus latiforus*, *Phyllostachys aurea* var. *albovariegada* and *Phyllostachys edulis*) were characterized on the basis of vegetative descriptors, pseudopetiole, sheath, ligule, gems and culm. The genetic divergence between the clones was estimated by the methods of grouping of Tocher and UPGMA with use of average Euclidean distance and the principal component in two-dimensional plane. Qualitative and quantitative descriptors proposed were efficient to differentiate the six species studied and quantify genetic diversity. The quantitative descriptor of sheath length was the largest contributor to differentiate the species studied.

Key words: *Bambusa*. *Drepanostachyum*. *Dendrocalamus*. *Phyllostachys*. Genetic diversity.

RESUMO - O bambu é um recurso genético com evidente potencial para utilização que vai desde a construção civil até a recuperação de áreas degradadas. Mesmo assim, os estudos de caracterização e avaliação envolvendo espécies de bambu são escassos e dificultam a definição de uma lista de descritores específica para melhor conhecer os diferentes gêneros e/ou espécies de bambu. Desse modo, objetivou-se neste trabalho propor e testar a eficiência de descritores qualitativos e quantitativos visando à caracterização fenotípica e ao estudo de diversidade genética entre seis espécies de bambu introduzidas no Brasil. Quinze descritores qualitativos e nove quantitativos foram propostos e testados em clones com um ano de plantio. Indivíduos pertencentes a seis espécies de bambu (*Bambusa vulgaris*, *Bambusa vulgaris* var. *vittata*, *Drepanostachyum falcatum*, *Dendrocalamus latiforus*, *Phyllostachys aurea* var. *albovariegada* e *Phyllostachys edulis*), foram caracterizados com base em descritores vegetativos de folha, pseudopecíolo, bainha, lígula, gemas e colmo. A divergência genética entre os clones foi estimada pelos métodos de agrupamento de Tocher e UPGMA, com emprego da distância Euclidiana média e pelos Componentes Principais no plano bidimensional. Os descritores qualitativos e quantitativos propostos foram eficientes para diferenciar as seis espécies estudadas e quantificar a diversidade genética. O descritor quantitativo de comprimento da bainha foi o que mais contribuiu para diferenciar as espécies estudadas.

Palavras-chave: *Bambusa*. *Drepanostachyum*. *Dendrocalamus*. *Phyllostachys*. Diversidade genética.

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INTRODUCTION

The bamboo is a member of the Poaceae family, included in the subfamily Bambusoideae, with 1,439 species (BAMBOO PHYLOGENY GROUP, 2012; SUNGKAEW *et al.*, 2009). Bamboos are distributed throughout the tropical and temperate areas of Asia, Australia, Africa and America (GUERREIRO; LIZARAZU, 2010).

Brazil has a great diversity of bamboo; thirty-three genera and around 250 species can be found, of which about 160 are considered to be endemic, with some that have not yet been formally described. In addition, over 20 species of bamboo have been introduced into Brazil (FILGUEIRAS *et al.*, 2013.), mainly during the colonial period and later by Japanese immigrants, most of these being species native to Asia (SILVA; PEREIRA; SILVA, 2011; TOMBOLATO; GRECO; PINTO, 2012).

There are many different uses for bamboo. Many species are grown as ornamental plants, while others are used to control erosion in degraded areas, as a source of raw material for the structure of housing, as forage, as a source of fibre for the pulp and paper industry and as a source of biomass for energy production; the edible shoots are used for food. In addition, bamboo serves as raw material for craftsmen, making it a useful resource in local economies (FILGUEIRAS *et al.*, 2013; FILGUEIRAS; GONÇALVES; 2004; RESENDE *et al.*, 2011).

Bamboos form a monophyletic group within the Poaceae family, and can be recognised by their morphological, anatomical, embryological, physiological, cytological, macromolecular and ecological characteristics. These characteristics are used by researchers to identify, qualify, characterise, name, classify and recommend uses for the various species that make up this rich and diverse group of plants (FILGUEIRAS; GONÇALVES, 2011). One of the more interesting features among bamboos is related to flowering. Flowering in most species of bamboo is irregular until the end of the vegetative stage, a stage that can last up to 120 years (AZZINI; CLARAMELLO; NAGAI, 1978). After flowering some species die, making study of the floral characteristics of the bamboo difficult; they also produce a small number of seeds (RAMANAYAKE, 2006).

Most current classifications of the bamboo depend on morphological characteristics (CLARK *et al.*, 2007), however few species have been properly documented, making it difficult to use phenotypic features. This is further compounded by the fact that taxonomic studies traditionally depend on inflorescence and floral morphology, since vegetative characteristics can be influenced by environmental conditions (SHALINI *et al.*, 2013; YEASMIN *et al.*, 2015).

Genetic diversity in the bamboo should be studied, providing data to investigate evolution of the bamboo through speciation (FILGUEIRAS; GONÇALVES, 2011). In addition, for bamboo to be seen as a viable alternative for its many uses, studies are necessary, so as to characterise and evaluate possible species for use in appropriate applications. However, studies into the characterisation and evaluation of bamboo species are scarce, and there is as yet no definitive list of morphological descriptors. The aim of this study therefore, was to propose and test the efficiency of qualitative and quantitative descriptors, with a view to the phenotypic characterisation and to the study of genetic diversity among six species of bamboo introduced into Brazil.

MATERIAL AND METHODS

Six accessions were used in the study, representing different species of bamboo (*Bambusa vulgaris*, *Bambusa vulgaris* var. v*ittata*, *Drepanostachyum falcatum*, *Dendrocalamus latiforus*, *Phyllostachys aurea* var. *albovariegada* and *Phyllostachys edulis*). All material was provided by the Biomudas Laboratory, which maintains a collection of bamboos at Venda Nova do Imigrante, in the State of Espírito Santo (ES), Brazil. Plantlets from each species were obtained by the division method, used in the propagation of bamboo, where parts were taken from the rhizome and culm, and planted in 30-litre pots containing Basaplant® Florestais substrate.

The experiment was carried out in a greenhouse of the Research Support Unit (UAP) of the Darcy Ribeiro North Fluminense State University (UENF), at Campos dos Goytacazes, in the State of Rio de Janeiro (RJ), located at 21º45' S and 41º20' W at an average altitude of 11 metres.

Morphological characterisation was performed for two plants per species after around one year of growth. These were properly identified using bamboo identification keys to confirm the species (SHIRASUNA, 2012; WONG, 2004).

Vegetative morphological characteristics which are noticeably variable, from such parts of the plant as the culm, branches, leaves, petioles, sheath, ligule and buds, were used to make up the proposed descriptors for the species being studied, resulting in 15 qualitative and nine quantitative characteristics (Tables 1 and 2). For the qualitative characteristics, which involved establishing the colour, a scale was used to determine colour intensity. The quantitative characteristics were measured with the aid of a digital calliper, metal tape measure and millimetre rule.

Table 1 - Proposed qualitative descriptors for the bamboo species: *B. vulgaris, B. vulgaris* var. *vittata, D. falcatum, D. latiforus, P. aurea* var. *albovariegada* and *P. edulis*.

Descriptor name	Abbreviation	Evaluation
Culm sheath colouration	SC	1: Green 2: Green-yellow 3: Green-white
Culm sheath wax	CSW	0: Absent 1: Present
Culm colour	CC	1: Green 2: Yellow-green 3: Green-green
Blade colour	BC	1: Green 2: Green-white
Presence of node characteristics	NC	0: Absent 1: Present
Auricle development	AD	1: Indistinct 2: Evident
Ligule development	LD	1: Indistinct 2: Evident
Internode spikes	IS	0: Absent 1: Present
Shape of culm	CS	1: Cylindrical 2: Crooked
Culm sheath pilosity	SP	1: Glabrous 2: Pilose
Internode pilosty	IP	1: Glabrous 2 Pilose
Blade pilosity	BPI	1: Glabrous 2: Pilose
Sheath blade position	BP	1: Erect 3: Hanging 5: Intermediate
Internode filling	IF	1: Hollow 2: Solid
Variation in culm colouration	VC	1: Uniform 2: Striped

Table 2 - Proposed quantitative descriptors for the bamboo species: *B. vulgaris, B. vulgaris* var. *vittata, D. falcatum, D. latiforus, P. aurea* var. *albovariegada* and *P. edulis.*

Descriptor name	Abbreviation	Evaluation
Plant height	PH	In metres, from the ground to the highest leaf on the plant
Length of internode	IL	In centimetres, measured between 2 nodes from the 1st to the 5th internode on the culm
Length of leaf	LL	In centimetres, from the base to the tip of the leaf blade in 5 leaves
Length of culm sheath	SL	In millimetres, from the base of the sheath to the start of the pseudopetiole in 5 sheaths
Length of pseudopetiole	LP	In millimetres, from the base of the pseudopetiole to the start of the blade in 5 leaves
Diameter of culm	CD	In millimetres, from the basal, median and apical regions of the first internode
Width of leaf	LW	In millimetres, from one side to the other in 5 leaves
Number of buds per node	NB	Counting the number of buds on the largest culm
Number of branches per bud	BB	Counting the number of emerging branches in 5 buds

Genetic divergence between the species, and multivariate analysis using the mean Euclidean distance as a measure of dissimilarity, were determined by Tocher clustering optimisation (RAO, 1952), mean hierarchical clustering between groups (UPGMA), and principal component analysis. All analyses of genetic divergence were performed using the GENES software (CRUZ, 2013).

RESULTS AND DISCUSSION

For the qualitative features, no variability was seen in the descriptors of node characteristics (NC), internode spikes (IS), shape of culm (CS), internode pilosity (IP), blade position (BP) and internode filling (IF). Therefore these descriptors did not contribute to differentiating between the species (Table 3).

Table 3 - Qualitative morphological characteristics in six bamboo genotypes, evaluated in a greenhouse

	B. vulgaris	*B. vulgaris vittata*	*D. falcatum*	*D. latiforus*	*P. aurea*	*P. edulis*
SC	Medium green	Medium green with yellow stripes	Light green	Medium green with yellow stripes	Medium green with white stripes	Medium green
CSW	Absent	Absent	Absent	Absent	Present	Absent
CC	Dark green	Yellow with dark green stripes	Light green	Medium green with dark green stripes	Medium green	Medium green
BC	Dark Green	Dark green	Light green	Dark green	Medium green with white stripes	Dark green
NC	Absent	Absent	Absent	Absent	Absent	Absent
AD	Evident	Indistinct	Evident	Evident	Indistinct	Evident
LD	Evident	Indistinct	Indistinct	Indistinct	Evident	Evident
IS	Absent	Absent	Absent	Absent	Absent	Absent
CS	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical	Cylindrical
SP	Glabrous	Pilose	Glabrous	Pilose	Pilose	Pilose
IP	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous	Glabrous
BPI	L: Present R: Absent E: Present	L: Present R: Present E: Present	L: Present R: Absent E: Present	L: Absent R: Present E: Present	L: Absent R: Absent E: Present	L: Present R: Present E: Present
BP	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate	Intermediate
IF	Hollow	Hollow	Hollow	Hollow	Hollow	Hollow
VC	Uniform	Striped	Uniform	Striped	Uniform	Uniform

Descriptors: culm sheath colouration (SC), culm sheath wax (CSW), culm colour (CC), blade colour (BC), presence of node characteristics (NC), auricle development (AD), ligule development (LD), internode spikes (IS), shape of culm (CS), culm sheath pilosity (SP), internode pilosity (IP), sheath blade pilosity (BPI) on the leaf blade (L), ribs (R) and edges (E), sheath blade position (BP), internode filling (IF) and variation in culm colour (VC)

For sheath colouration (SC), the species *B. vulgaris vitatta* and *D. latiforus* presented light green sheaths with yellow stripes. The species P. *aurea albovariegada* displayed sheaths of a green colouration with white stripes. The species *B. vulgaris* and *P. edulis* had medium green sheaths, and *D. falcatum*, light green, this being a feature that facilitated differentiation between the species. The presence of sheath wax (CSW) was only seen in *P. aurea albovariegada* (Table 3).

The species with a culm colouration (CC) of two colours are *B. vulgaris vitatta*, with yellow culms with dark green stripes, and *D. latiforus*, with medium green culms with dark green stripes. The species *P. aurea albovariegada* and *P. edulis* had medium green culms, *D. falcatum* had light green culms and *B. vulgaris* dark green culms (Table 3).

For the descriptor of leaf-blade colour (BC), the species *D. falcatum* displayed clear green leaf blades, in the species *B. vulgaris, B. vulgaris vitatta, latiforus D.* and *P. edulis* the blades had a dark green colouration, and in *P. aurea albovariegada* they were medium green with white stripes (Table 3). This characteristic for leaves of more than one colour encourages the use of the species as an ornamental plant. For the descriptor of auricle development (AD), evident auricles were seen in the species *D. falcatum*, *B. vulgaris D. latiforus* and *P. edulis*. At the junction of the blade and the sheath is the external ligule (LD), situated on the abaxial surface, and which may or may not be evident. Only the species *B. vulgaris*, *P. edulis* and *P. aurea albovariegada* displayed evident ligules (Table 3).

For the descriptor of sheath pilosity (SP), the species *D. falcatum* and *B. vulgaris* have glabrous sheaths, while those of the remaining species are pilose (Table 3).

Pilosity of the leaf blade (BPI) was evaluated for the blade (L), the ribs (R) and the borders (B). Pilosity was seen in the borders of the leaf blade in all the species under study, with the species *B. vulgaris vittata* and *P. edulis* presenting pilosity throughout the leaf-blade area. In the species *D. falcatum* and *B. vulgaris*, pilosity was seen on the blade, and in *D. latiforus*, pilosity was seen on the ribs (Table 3).

The quantitative characteristics contributed to differentiating between the species (Table 4).

Table 4 - Quantitative morphological characteristics in six bamboo genotypes, evaluated in a greenhouse

Genotype	PH	IL	LL	SL	LP	CD	LW	NB	BB
B. vulgaris	2.93	23.65	18.18	58.61	2.91	15.09	32.10	2.1	2.1
B. vulgaris vittata	3.05	23.76	21.88	62.85	3.39	22.45	31.85	3.2	3.2
D. falcatum	1.40	10.94	10.60	41.82	0.99	7.22	7.15	0.9	8.0
D. latiforus	2.84	23.38	16.66	65.95	3.04	13.00	29.46	4.6	4.6
P. aurea albovariegada	0.41	2.64	10.94	31.66	2.29	6.29	12.38	0.9	3.4
P. edulis	0.62	3.86	11.80	29.36	4.28	11.34	16.40	1.2	1.2

Plant height (PH) (m), length of internode (IL) (cm), length of leaf (LL) (cm), length of culm sheath (SL) (mm), length of pseudopetiole (LP) (mm), average diameter of culm (CD) (mm), width of leaf (LW) (mm), number of buds per node (NB) and number of branches per bud (BB)

The method proposed by Singh (1981) made it possible to estimate the relative contribution of each quantitative descriptor to the genetic diversity between species. The quantitative characteristic that least contributed to differentiate between the species under study was seen to be plant height (PH) (0.22%). The characteristics with the greatest contribution were the length of the culm sheath (SL) (39.88%), width of the leaf (LW) (18.24%) and length of the internodes (IL) (15.4%) (Table 5).

Despite the low contribution of plant height to divergence, this descriptor cannot be ignored, as it is decisive when utilising the species. As pointed out by Wahab *et al*. (2010), plant height, number of internodes per culm, internode length and culm diameter are important factors in determining the most appropriate use for each species of bamboo.

When evaluating genetic divergence according to the values obtained for dissimilarity, the greatest genetic distance was between the species *B. vulgaris vittata* and *P. aurea albovariegada* (Table 6). The species involved belong to different genera and subtribes, which explains this result; the genus *Bambusa* belongs to the subtribe Bambusinae, while the genus *Phyllostachys* belongs to the subtribe Shibataeinae (DAS *et al*., 2008). The smallest genetic distance was found between the species *B. vulgaris* and *D. latiforus* (Table 6).

The Tocher method, when applied to qualitative data, allows for the formation of clusters based on the dissimilarity expressed by the divergence matrix obtained with the index '1–C', where 'C' is the concordance of values expressed during comparison of the species. Thus, with the qualitative characteristics, the formation of three groups was seen, being a more restrictive

Table 5 - Relative contribution of quantitative characteristics to genetic divergence between six bamboo genotypes, using a method proposed by Singh (1981)

Caracteristic	Value %	Accumulated %
Length of culm sheath	39.88	39.88
Width of leaf	18.24	58.12
Length of internodes	15.70	73.82
Culm diameter at the basal region of the 1st internode	5.72	79.54
Culm diameter at the median region of the 1st internode	5.69	85.23
Culm diameter at the apical region of the 1st internode	5.29	90.52
Average culm diameter	4.58	95.1
Length of leaf	3.24	98.34
Number of branches per node	0.86	99.2
Number of buds per node	0.33	99.53
Length of petiole	0.25	99.75
Plant height	0.22	100.00

Table 6 - Measures of dissimilarity between six bamboo genotypes based on the mean Euclidean distance

Genotype	2	3	4	5	6
1. *D. falcatum*	1.55	2.11	1.48	1.32	0.77
2. *B. vulgaris*		0.81	0.63	1.23	1.50
3. *B. vulgaris vittata*			1.04	1.73	2.14
4. *D. latiforus*				1.39	1.54
5. *P. edulis*					0.78
6. *P. aurea albovariegada*					

method due to the use of discrete variables. Group 1 comprised the species *D. falcatum*, *B. vulgaris* and *P. edulis*. Group 2 included the species *B. vulgaris vittata* and *D. latiforus*. Finally, group 3 consisted of the species *P. aurea albovariegada* (Table 7).

Four groups were formed for the quantitative characteristics. Group 1 was composed of the species *B. vulgaris* and *D. latiforus*, group 2 of *D. falcatum* and *P. aurea albovariegada*, group 3 of *P. edulis* and group 4 of *B. vulgaris vittata* (Table 8).

The dendrogram of genetic similarity between the six bamboo genotypes, obtained by UPGMA, and based on the dissimilarity matrix of the qualitative data (Figure 1), showed that with a 40% cut it was possible to form four groups. The dendrogram based on the dissimilarity matrix of the quantitative data (Figure 2) showed that with 40% of the data, five groups were formed.

Principal component analysis reduced the nine quantitative characteristics to two principal

Table 7 - Groups of bamboo genotypes established by the Tocher method, based on the dissimilarity expressed by the mean Euclidean distance obtained from qualitative characteristics and descriptors that facilitated the formation of groups

Group	Genotype	Descriptor
1	*D. falcatum, B. vulgaris and P. edulis*	Culm sheath wax, leaf blade colour, node characteristics, auricle development, internode spikes, shape of culm, internode pilosity, leaf-blade position and variation in culm colour
2	*B. vulgaris vitatta and D. latiforus*	Culm sheath colour, culm sheath wax, leaf-blade colour, node characteristics, ligule development, internode spikes, shape of culm, culm sheath pilosity, internode pilosity, leaf blade position, internode filling and variation in culm colour
3	*P. aurea albovariegada*	Culm sheath colour, culm sheath wax and leaf blade colour

Table 8 - Groups of bamboo genotypes established by the Tocher method, based on the dissimilarity expressed by the mean Euclidean distance obtained from quantitative characteristics that most contributed to the formation of groups

Group	Genotype	Descriptor
1	*B. vulgaris e D. latiforus*	Plant height, length of internodes and culm diameter
2	*D. falcatum e P. aurea albovariegada*	Length of leaf and number of buds per node
3	*P. edulis*	Length of pseudopetiole and culm diameter
4	*B. vulgaris vitatta*	Length of leaf and culm diameter

Figure 1 - Dendrogram of the genetic similarity between six bamboo genotypes, obtained by UPGMA, and based on the dissimilarity matrix of the qualitative data

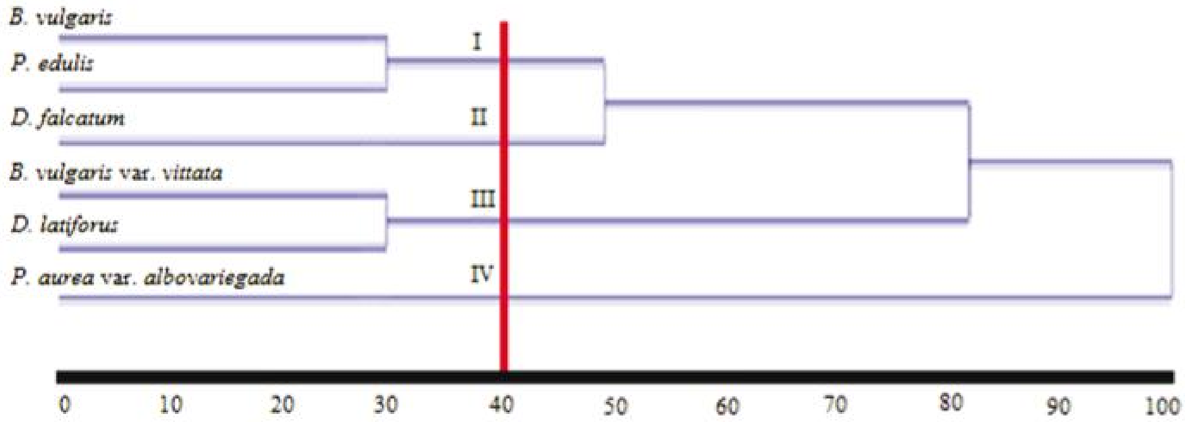
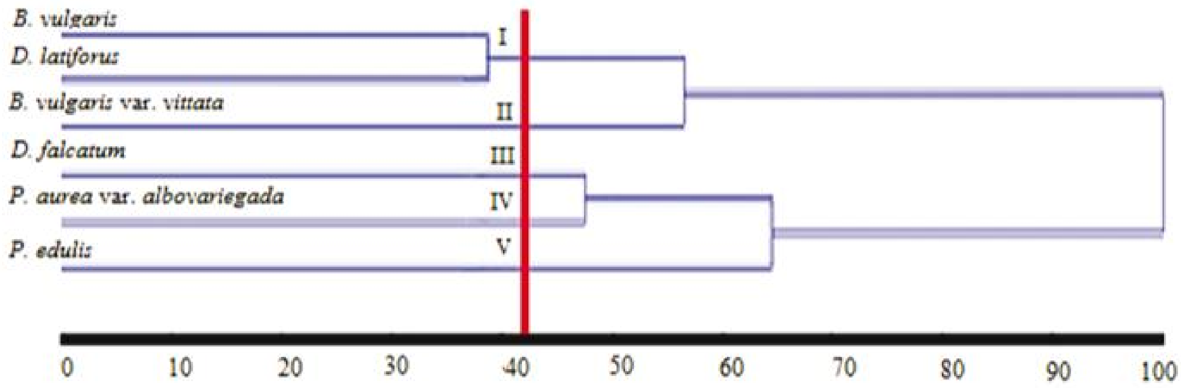


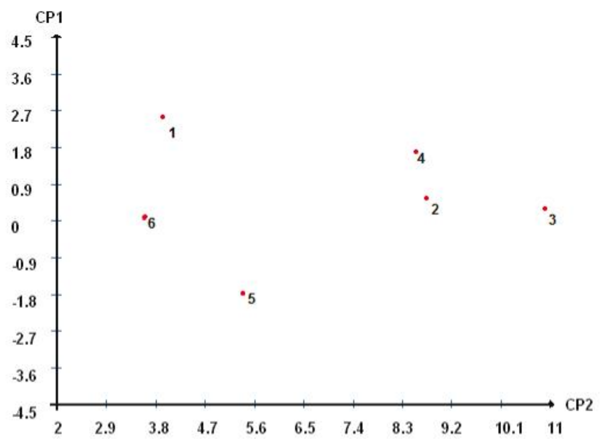
Figure 2 - Dendrogram of the genetic similarity between six bamboo genotypes, obtained by UPGMA, and based on the dissimilarity matrix of the quantitative data



components (PC1 and PC2), which explained 91.44% of the total variance between the six species. The greatest proximity was seen between the species *D. latiforus* (4) and *B. vulgaris* (2). The greatest distance was seen between the species *P. aurea albovariegada* (6) and *B. vulgaris vittata* (3) (Figure 3).

Based on the vegetative morphological descriptors, the species *D. latiforus* and *B. vulgaris* were seen to be very close. At the stage evaluated in this study, the two species are very similar. This was also noted by Sun, Xia and Lin (2005) where, by using ribosomal DNA as molecular markers in phylogenetic analysis, the species *Bambusa* and *Dendrocalamus* were allocated to the same group. According to Li (1997), there are similarities between many species of the genera *Bambusa* and *Dendrocalamus*, although the relationship between these genera is still not well understood.

Figure 3 - Principal component analysis (PC1 and PC2), with 91.44% of accumulated variance, between six bamboo genotypes: (1) *D. falcatum*, (2) *B. vulgaris*, (3) *B. vulgaris vittata*, (4) *D. latiforus*, (5) *P. edulis* and (6) *P. aurea albovariegada*, based on quantitative characteristics



Classical systems for classifying bamboo are based on vegetative and reproductive morphological characteristics (DAS *et al*., 2007), although the reproductive characteristics of these plants often prevent such classification. Vegetative descriptors therefore, although they may be influenced by environmental conditions, are reliable and should be used in diversity studies.

In this work, high genetic diversity was seen between the six species under study using morphological descriptors; this was also observed by Das *et al*. (2007), who found high genetic diversity between 15 species of bamboo in India. Also, in studies involving different individuals of the same species, *Bambusa Tulda*, Bhattacharya *et al*. (2006) found little genetic diversity as would be expected. Further studies involving a large number of bamboos from different regions are therefore required to achieve a better understanding of their genetic diversity.

CONCLUSIONS

1. The proposed qualitative and quantitative descriptors were efficient in differentiating the species *B. vulgaris*, *B. vulgaris* var. *vittata*, *D. falcatum*, *D. latiforus*, *P. aurea* var. *albovariegada* and *P. edulis*;
2. The species showed high genetic diversity;
3. The largest contributor in differentiating between the species under study was the quantitative descriptor for length of sheath;
4. The species with the greatest genetic proximity were *D. latiforus* and *B. vulgaris*. The greatest distance was seen between the species *P. aurea albovariegada* and *B. vulgaris vittata.*

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